

FIG. 1A: Full length Intron A = 820 bp

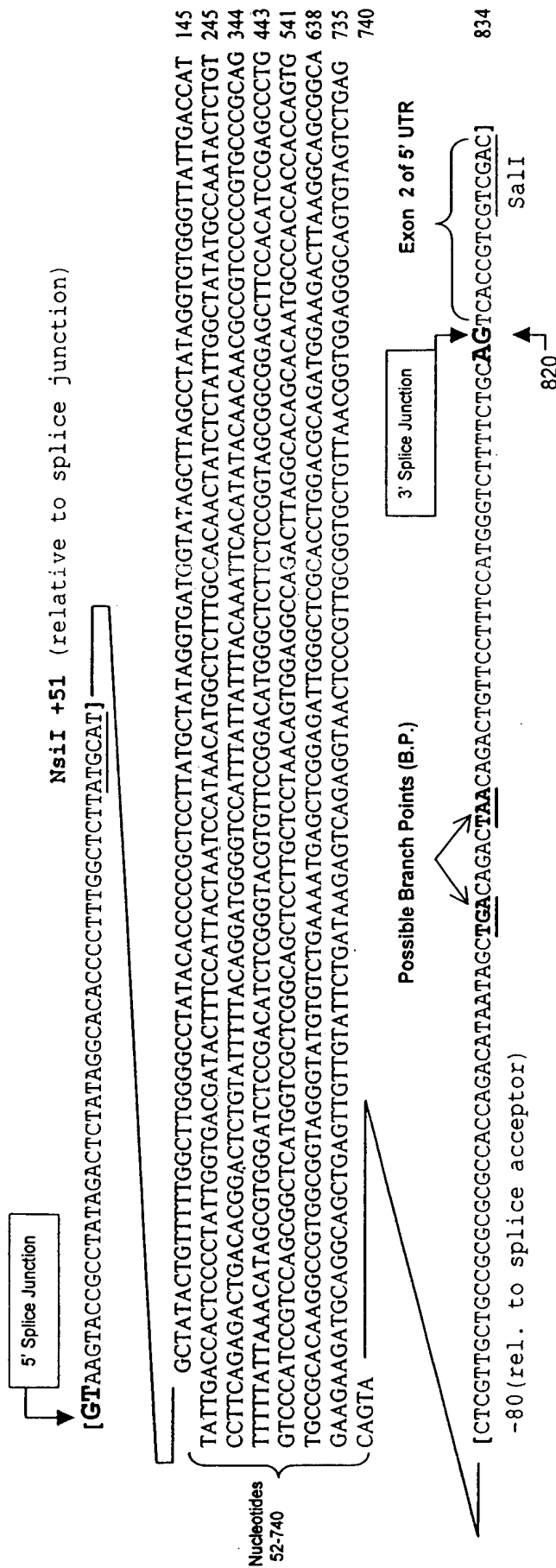


FIG. 1B: Synthetic Oligo for substitution of nucleotides 52-740 of Intron A

[ATGCATCTCGTTGCTGCCGCGCGCCACCAGACATAATCGCTGACACACTGACAGACTGTTCCCTTTCTTCTTTTTCAGTACCCGTCGTCGAC]

NsiI -80

Changed to B.P. consensus

Changed to polypyrimidine consensus

SalI

FIG. 1C: Deletion Mutant pCON3 Intron: 132 bp

NsiI

[GTAAGTACCGCCTATAGACTCTATAGGCACACCCCTTTGGCTCTTATGCATCTCGTTGCTGCCGCGCGCCACCAGACATAATCGCTGACACACTGA]

Branch Point

CAGACTGTTCCCTTTCTTTTTCAGTACCCGTCGTCGAC]

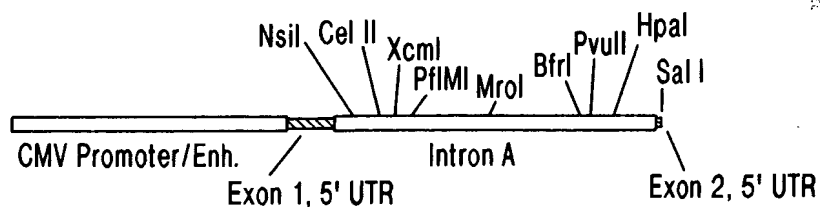
Polypyrimidine Tract

SalI

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FIG. 2

Deletions Made Within Intron A of CMV IE1



DELETION LENGTH*

Nsi-Cel II	70 bp	
Nsi-XcmI	113 bp	
Nsi-PflMI	150 bp	
Nsi-MroI	345 bp	
Nsi-BfrI	578 bp	
Nsi-PvuII	609 bp	
Nsi-HpaI	663 bp	

Nsi - X Truncations

DELETION LENGTH*

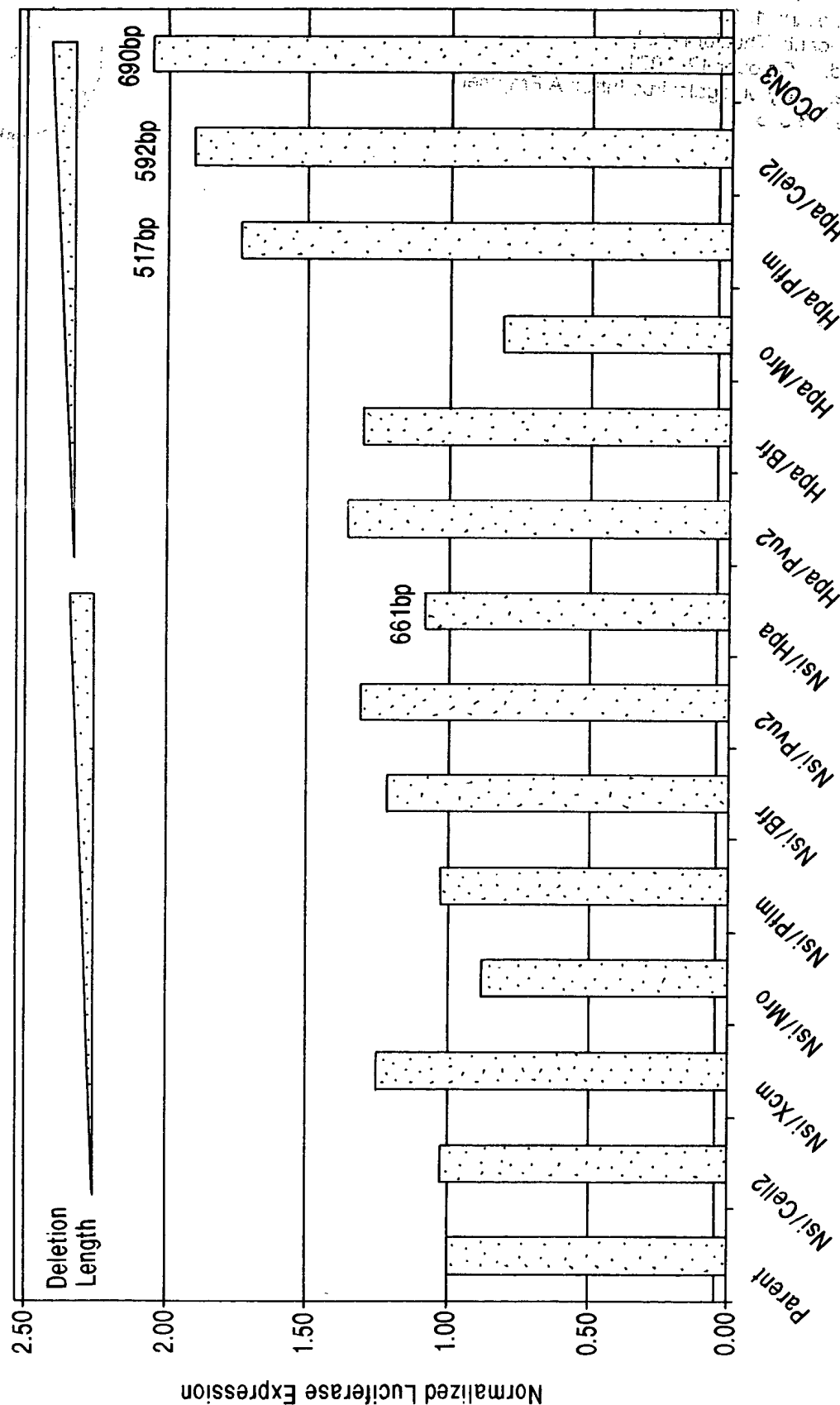
Hpa-PvuII	54 bp	
Hpa-BfrI	80 bp	
Hpa-MroI	314 bp	
Hpa-PflMI	516 bp	
Hpa-Cel II	590 bp	

Hpa - X Truncations

* Following restriction enzyme digestion, building, religation

FIG. 3

Intron A Internal Deletion Mutants (Transiently-Transfected 293 cells)



Construct I.D.

FIG. 4

1. Wild Type Rabbit β -Globin Sequence

GTTGGTATCCTTTTACAGCACAACTTAATGAGACAGATAGAAACTGGTCTTGTAGAAACA
Splice Donor

GAGTAGTCGCCTGCTTTTCTGCCAGGTGCTGACTTCTCTCCCCTGGGCTGTTTTCATTTTCTCAG
Branch Pt. Polypyrimidine Tract

FIG. 5A

2. Optimized Rabbit β -Globin Sequence

GTAAGTATCCTTTTACAGCACAACTTAATGAGACAGATAGAAACTGGTCTTGTAGAAACA
Splice Donor

GAGTAGTCGCCTGCTTTTCTGCCAGGTACTAACTTCTCTCCCCTCTCCTCTTTTCTTTTCTGCAG
Branch Pt. Polypyrimidine Tract

FIG. 5B

Rabbit β -Globin Intron

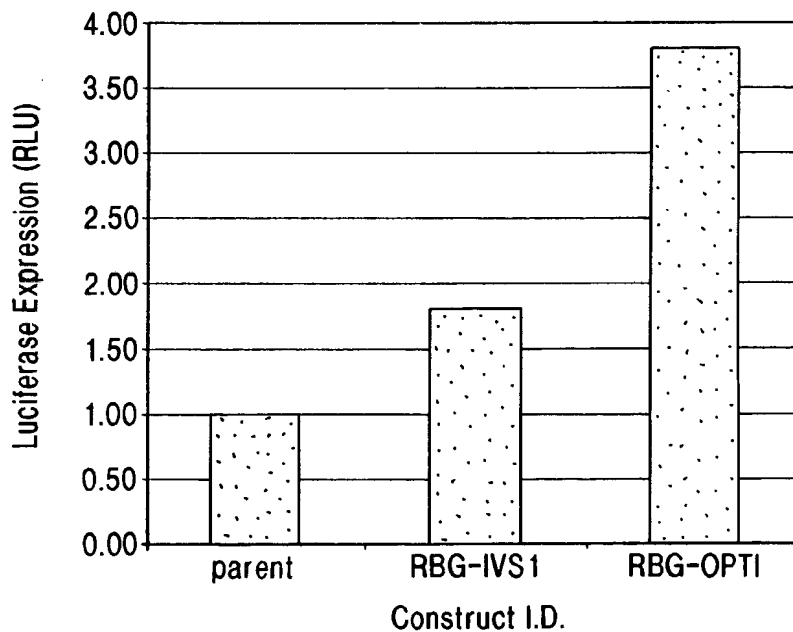
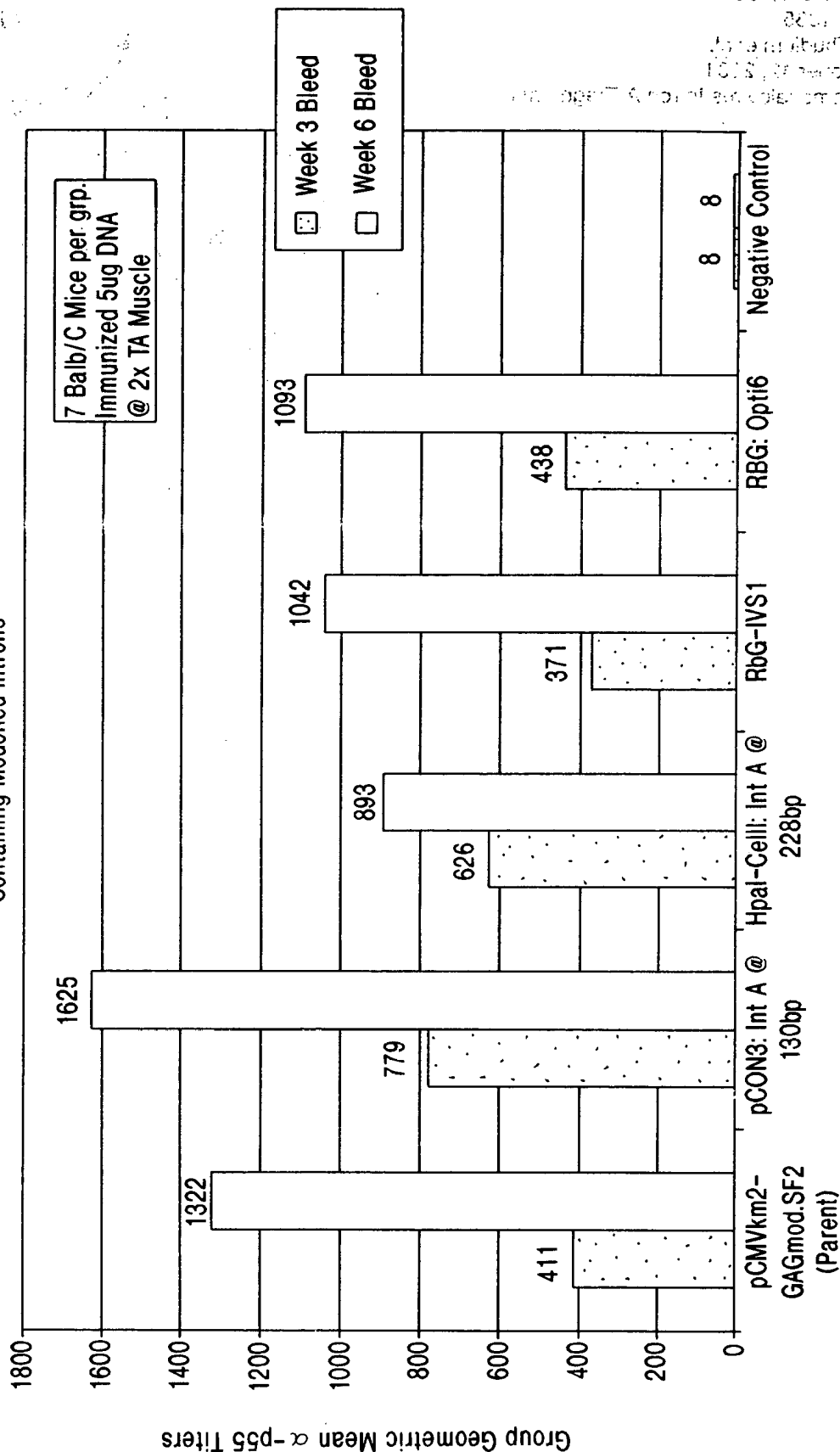


FIG. 6

In Vivo Immunogenicity of Plasmid Vectors Containing Modified Introns



Immunizing Vector DNA

FIG. 7

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